Effect of cholera toxin on ileal water and solute transport after resection of the proximal small intestine in the rat*

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SUMMARY Intestinal adaptation after extensive small bowel resection results in mucosal hypertrophy and an increased capacity of the remaining small intestine to absorb solutes and water. We tested the ability of the adapted rat ileum to respond to a secretory stimulus, cholera toxin. Six weeks after 50% jejunal resection (short gut) or sham operation water and solute transport were measured in a 16 cm segment of ileum before and after exposure to cholera toxin in a single pass *in vivo* perfusion system. During the control periods absorption of glucose, acetate and water per unit length of intestine was significantly greater in short gut animals ($P < 0.05$ to 0.001). After exposure to cholera toxin absorption of glucose and acetate was significantly reduced in both groups ($P < 0.05$ to 0.01). Sodium and chloride secretion and net change in water movement in response to cholera toxin were significantly greater ($P < 0.05$ to 0.01) in short gut animals. Generally the differences between short gut and sham operation animals disappeared when the data were normalised for mucosal weight. Chloride secretion per gram mucosa was less in short gut animals ($P < 0.001$). The data indicate that the adapted small bowel is not only capable of enhanced absorption but also of enhanced net secretion in response to cholera toxin. The changes reflect the increased number of enterocytes per unit length of intestine after intestinal adaptation.

Intestinal adaptation after extensive small bowel resection is characterised by increased villous height,¹⁻³ crypt cell proliferation,^{3,4} and enhanced ability for solute^{1,3,5} and water⁶ absorption in the remaining small intestine. As the mucosal surface is enlarged by the process of adaptation, one would expect that the remaining intestine is not only capable of enhanced absorption but that it should also respond with enhanced secretion of electrolytes and water when exposed to a secretory stimulus. Here we report studies of water and solute transport in the adapted small bowel of the rat before and after exposure to cholera toxin. The results are

compared with the response in sham operated animals.

Methods

ANIMAL MODEL

Male Sprague-Dawley rats (Holtzman Co., Madison, WI) weighing 235-260 g, underwent surgery under general anaesthesia with pentobarbitone and ether. The operation was either a simple laparotomy (sham operated rats) or a resection of 50% of the small intestine starting at the ligament of Treitz with an end-to-end jejunoileal anastomosis (short gut rats). Postoperatively the animals were maintained on a full-strength liquid element diet (Flexical, Meade-Johnson, Evansville, Indiana) with free access to water. Six weeks after surgery perfusion studies were performed under general anaesthesia after an overnight fast. A 16 cm segment of small intestine with its distal end 5 cm proximal to the ileocaecal valve was isolated, with its blood supply

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intact, cannulated, rinsed with 0.9% NaCl, and returned to the abdomen. The segment was perfused in a single pass at 1 ml/min. The temperature of the animals was maintained at 37°C with a heat lamp controlled by a rectal thermometer.

EXPERIMENTAL DESIGN AND COMPOSITION OF PERFUSION SOLUTIONS

Each experiment consisted of five one hour perfusion periods. Two test solutions, A and B, were perfused for one hour before and after exposure of the test segment to cholera toxin. The perfusion sequence, therefore, was always A-B—exposure to cholera toxin—A-B; each one hour perfusion period consisted of a 20 minute equilibration period and two 20 minute sampling periods. Solution A contained in mmol/l: Na 50, K 2.5, Cl 2.5, acetate 50, polyethylene glycol 4000 (PEG-4000) 10 g/l, ^{14}C -PEG (New England Nuclear, Boston, Mass.) 10 $\mu\text{Ci/l}$ and mannitol to an osmolality of 300 mOsm/l. pH was 7.4. Solution B differed from solution A by the addition of 10 mmol/l glucose and a corresponding reduction in mannitol concentration. Exposure to cholera toxin was accomplished by recirculating 25 ml 0.9% saline containing 250 μg cholera toxin (Wyeth, NIH Lot 0172) at 1 ml/min for one hour. After completion of the experiment the test segments were excised, weighed and the *ex vivo* length measured under tension of a 5 g weight. The mucosa of the central 4 cm of each test segment was stripped and weighed with the assumption that it was representative of the entire test segment.

ANALYTICAL METHODS

PEG was determined as ^{14}C -PEG.⁷ For the isotope determination, 1 ml of the perfusion solution or collected samples was mixed with 10 ml of a scintillation of cocktail composed of toluene and emulsifier (Ready Solv HP, Beckman Instruments, Inc., Fullerton, CA) and counted in a liquid scintillation counter (Beckman, model LS-255). Quench correction was made by external standardisation. Sodium and potassium were measured by flame photometry, chloride by electrometric titration with a silver nitrate solution and glucose by the glucose oxidase method (Boehringer Mannheim Corp., New York, NY). Acetate was measured by gas liquid chromatography⁸ (Gaschromatograph: Varian 3700 with flame ionisation detector). To 1 ml of the samples 50 μl 400 mM sodium isobutyrate were added as an internal standard. After vortex mixing, 2 drops of concentrated HCl (approximately 500 μmol) were added and the sample immediately closed with a stopper. Analysis was carried out on a glass column (200 cm long, 2 mm internal diameter) packed with

10% AT-100 on 80/100 Chromasorb W-AW (Alltech Assoc., Arlington Heights, IL). Column temperature was 125°C (isothermal). Peak areas were determined with a digital integrator. Linear dose responses for acetate were obtained under these conditions over a range from 0–100 mmol/l.

CALCULATIONS AND STATISTICAL ANALYSIS

Net water and solute movements were calculated by standard formulas using the measured PEG and solute concentrations.⁹ Steady state conditions were confirmed by comparing the PEG concentrations during the first and second 20 minute sampling periods within each perfusion period. No significant differences were observed. The mean of the two consecutive 20 minute sampling periods, therefore, was used as one data point. The data were normalised for segment length of intestine or mucosal weight. Paired and unpaired *t* tests were used for statistical analysis. All data are reported as means $\pm$ SE.

Results

Sham operated ($n=5$) and short gut ($n=6$) animals gained 35 ± 2 g and 33 ± 3 g respectively during the six weeks after surgery and weighed 285 ± 3 g and 278 ± 6 g at the time of study. The *ex vivo* length of the test segments was 16.2 ± 1.5 cm in the sham operated group and 17.0 ± 0.7 cm in the short gut group (NS). The test segments weighed 47.4 ± 1.6 mg/cm in the sham operated animals and 76.3 ± 3.8 mg/cm in the short gut animals ($P<0.005$). The mucosal weight was 14.2 ± 0.8 mg/cm and 26.5 ± 1.9 mg/cm respectively ($P<0.005$).

NET WATER MOVEMENT (Table 1)

Short gut animals absorbed water at a faster rate than sham operated animals under control conditions ($P<0.05$ – 0.025). Exposure to cholera toxin resulted in net fluid secretion under all test conditions. During perfusion of solution B net fluid secretion in short gut animals was greater than in sham operated animals but the difference failed to reach statistical significance ($P<0.1$). The total change in water movement under the influence of cholera toxin was significantly greater in short gut animals during perfusion of solution A ($P<0.05$) and B ($P<0.01$).

ABSORPTION OF GLUCOSE AND ACETATE (Table 2)

As the changes in solute absorption induced by cholera toxin were essentially the same for solution A and B we report here only the data obtained during perfusion of solution B. Glucose and acetate

Table 1 Net water movement in sham operated and short gut rats in response to cholera toxin ($\mu\text{l}/\text{cm}/\text{h}$; mean $\pm$ SE)*

	Sham (n=5)		Short gut (n=6)	
	Solution A	Solution B	Solution A	Solution B
Control	9 $\pm$ 12	17 $\pm$ 5	40 $\pm$ 12†	49 $\pm$ 12†
Post-cholera toxin	-91 $\pm$ 7 §	-53 $\pm$ 7 §	-99 $\pm$ 15§	-78 $\pm$ 14§
Net change in water movement	100 $\pm$ 16	69 $\pm$ 11	139 $\pm$ 10†	127 $\pm$ 15*

* Minus signs indicate net secretion.

† P < 0.05 vs sham.

‡ P < 0.025 vs sham.

§ P < 0.0025 vs control.

* P < 0.01 vs sham.

16 cm of ileum were perfused with solution A (no glucose) and B (10 mM glucose) for 1 hour before and after exposure to cholera toxin (one hour).

Table 2 Net solute transport in sham operated and short gut rats in response to cholera toxin ($\mu\text{mol}/\text{cm}/\text{h}$; mean $\pm$ SE)*

Solute concn in perfusion solution (mM)		Sham (n=5)		Short gut (n=6)	
		Control	Post cholera toxin	Control	Post cholera toxin
Glucose	10	5.0 $\pm$ 0.9	3.6 $\pm$ 0.9†	7.7 $\pm$ 0.6§	5.4 $\pm$ 0.6†
Acetate-	50	14.4 $\pm$ 1.5	8.1 $\pm$ 1.1†	24.2 $\pm$ 1.3*	17.3 $\pm$ 1.4†*
Na+	50	2.4 $\pm$ 2.9	-13.8 $\pm$ 2.6†	1.1 $\pm$ 2.8	-22.6 $\pm$ 1.9†§
K+	2.5	-0.5 $\pm$ 0.1	-8.1 $\pm$ 0.7†	0.8 $\pm$ 0.1	-9.4 $\pm$ 1.2†
Cl-	2.5	-8.1 $\pm$ 0.7	-15.7 $\pm$ 0.7†	-9.4 $\pm$ 1.2	-19.3 $\pm$ 1.2†§

* Minus signs indicate net secretion.

† P < 0.01 vs control.

‡ P < 0.05 vs control.

§ P < 0.05 vs sham.

* P < 0.001 vs sham.

Experimental conditions as in Table 1. The data were obtained during perfusion of solution B.

absorption during the control periods was significantly greater in short gut animals than in sham operated animals ($P < 0.05$ and $P < 0.001$ respectively). When normalised for mucosal weight, glucose absorption was $0.30 \pm 0.03 \mu\text{mol}/\text{mg}/\text{h}$ in short gut animals and $0.37 \pm 0.07 \mu\text{mol}/\text{mg}/\text{h}$ in sham operated animals (NS). Acetate absorption normalised for mucosal weight was $0.92 \pm 0.05 \mu\text{mol}/\text{mg}/\text{h}$ and $1.05 \pm 0.15 \mu\text{mol}/\text{mg}/\text{h}$ respectively (NS). After exposure to cholera toxin glucose absorption decreased significantly in sham operated ($P < 0.01$) and short gut animals ($P < 0.01$). Similarly, acetate absorption decreased significantly in sham operated ($P < 0.05$) and short gut animals ($P < 0.01$). The glucose concentration in the effluent in sham operated rats was $8.1 \pm 0.2 \text{ mM}$ in the control period and $8.3 \pm 0.2 \text{ mM}$ after exposure to cholera toxin. The corresponding data in short gut animals were at $7.5 \pm 0.1 \text{ mM}$ and $7.8 \pm 0.1 \text{ mM}$ respectively.

ELECTROLYTE TRANSPORT (Table 2)

Under control conditions, sodium absorption was equal in both groups. After exposure to cholera toxin net sodium secretion was significantly greater in the short gut animals ($P < 0.02$). When sodium transport was normalised for mucosal weight, sodium absorption was $0.18 \pm 0.22 \mu\text{mol}/\text{mg}/\text{h}$

in sham operated and $0.02 \pm 0.11 \mu\text{mol}/\text{mg}/\text{h}$ in short gut animals (NS). The net secretion rates for sodium after exposure to cholera toxin were $-0.97 \pm 0.19 \mu\text{mol}/\text{mg}/\text{h}$ in short gut and $-0.87 \pm 0.1 \mu\text{mol}/\text{mg}/\text{h}$ in sham operated animals (NS). There was no difference between short gut and sham operated animals with respect to the net potassium movement. After exposure to cholera toxin, potassium absorption changed to potassium secretion ($P < 0.01$). Both groups of animals secreted chloride under control conditions to an equal degree. After exposure to cholera toxin, chloride secretion increased. The net secretion rate was significantly greater in the short gut animals ($P < 0.05$). When the data were normalised for mucosal weight, chloride secretion after exposure to cholera toxin was $-1.11 \pm 0.02 \mu\text{mol}/\text{mg}/\text{h}$ in sham operated animals and $-0.74 \pm 0.06 \mu\text{mol}/\text{mg}/\text{h}$ in short gut animals ($P < 0.001$).

Discussion

The animals were truly adapted as indicated by the increased mucosal weight and the increased absorption of glucose, acetate, and water. The composition of the perfusion solutions was chosen with a second set of experiments in mind, which are not part of this report. The rather low sodium concentration

in the perfusion solutions, however, distorted the outcome of the studies somewhat. It resulted in low sodium absorption rates and chloride secretion¹⁰ under control conditions and abolished any differences in sodium absorption between short gut and sham operated animals that might have been otherwise apparent. Because the perfusion solutions contained large amounts of readily absorbable organic solutes, water absorption was supported by the absorption of acetate¹¹ and glucose¹². Thus, the increased absorption of glucose and acetate in the short gut animals explains the enhanced absorption of water in this group. As solute transport followed a downhill concentration gradient, no concomitant changes in sodium absorption occurred.¹³

The observation that glucose and acetate absorption decreased after exposure to cholera toxin is at variance with earlier reports which state that glucose absorption is not affected in the presence of fluid secretion induced by cholera toxin^{14, 15}. The results reported here are not an artefact due to the composition of the perfusion solutions because we observed similar results when we perfused solutions containing 150 mM NaCl.¹⁶ In addition, Rhode and Chen¹⁷ observed a reduction in the absorption of arabinose and urea in the presence of fluid secretion induced by cholera toxin.

The experiments demonstrate that the adapted small bowel is not only capable of increased solute and water absorption, but that it can also respond with increased sodium and chloride secretion to a secretory stimulus, such as cholera toxin. As is true for enhanced solute absorption,^{3, 5} this effect reflects the increased number of enterocytes, as the difference between sham operated and short gut animals generally disappears when the data are normalised for mucosal weight. The reduced response in chloride secretion per mg mucosal weight in the short gut animals is more difficult to explain. In view of the artificially low chloride concentration a conclusive statement is not possible.

Although the greater secretion rates for sodium and chloride demonstrate the enhanced capacity of the adapted small bowel for electrolyte secretion in response to cholera toxin, net secretion rates for water in the short gut animals were not significantly different from the controls. This blunting of the net secretory response is probably the result of the composition of our perfusion solutions, which provided large amounts of absorbable solutes which enhanced water absorption.^{11, 12} This interpretation is supported by the fact that solute absorption after cholera toxin remained greater in the short gut animals and by the fact that the net

change in water movement in the response to cholera toxin was also significantly greater in these animals (Table 1). The studies, therefore, provide evidence that the adapted small bowel is not only capable of enhanced absorption but also of enhanced electrolyte and fluid secretion in response to cholera toxin and, presumably, other secretory stimuli.

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